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(54) Title: LUNG CANCER MARKER

(57) Abstract

The present invention discloses an isolated and purified nucleic acid sequence and corresponding amino acid sequence to a novel protein specific for human lung cancer cells. This gene is expressed at a much higher level in these cells than in normal lung cells, other normal tissues and other tumor cell lines tested. Also disclosed are three additional recombinant forms of this gene and protein, in the first two cases a membrane spanning region is removed and in the third case an amino acid is changed by *in vitro* mutagenesis. Also disclosed are nucleic acid probes for the detection of lung cancer cells from tissue biopsy and body fluids such as serum, sputum and bronchial washings. A method for expressing the antigen in a host cell and its subsequent use as an immunogen in antibody production for test applications is described. An ELISA test to measure shed antigen present in patient samples as well as an enzyme test to measure activity in specimens also is described.

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LUNG CANCER MARKER

TECHNICAL FIELD

The invention relates to genes and proteins specific for certain cancers and methods for their detection.

BACKGROUND OF THE INVENTION

Lung cancer is the most common form of cancer in the world. Estimates for the year 1985 indicate that there were about 900,000 cases of lung cancer worldwide. (Parkin, et al., "Estimates of the worldwide incidence of eighteen major cancers in 1985," *Int J Cancer* 1993; 54:594-606). For the United States alone, 1993 projections placed the number of new lung cancer cases at 170,000, with a mortality of about 88%. (Boring, et al., "Cancer statistics," *CA Cancer J Clin* 1993; 43:7-26). Although the occurrence of breast cancer is slightly more common in the United States, lung cancer is second behind prostate cancer for males and third behind breast and colorectal cancers for women. Yet, lung cancer is the most common cause of cancer deaths.

The World Health Organization classifies lung cancer into four major histological types: (1) squamous cell carcinoma (SCC), (2) adenocarcinoma, (3) large cell carcinoma, and (4) small cell lung carcinoma (SCLC). (The World Health Organization, "The World Health Organization histological typing of lung tumours," *Am J Clin Pathol* 1982; 77:123-136). However, there is a great deal of tumor heterogeneity even within the various subtypes, and it is not uncommon for lung cancer to have features of more than one morphologic subtype. The term non-small cell lung carcinoma (NSCLC) includes squamous, adenocarcinoma and large cell carcinomas.

Typically, a combination of X-ray and sputum cytology is used to diagnose lung cancer. Unfortunately, by the time a patient seeks medical help for their symptoms, the cancer is at such an advanced state it is usually incurable. *Cancer Facts and Figures (based on rates from*

NCI SEER Program 1977-1981), New York: American Cancer Society, 1986). Routine large-scale radiologic or cytologic screening of smokers has been investigated. Studies concluded that cytomorphological screening did not significantly reduce the mortality rate from lung cancer and was not recommended for routine use. ("Early lung cancer detection: summary & conclusions," *Am Rev Respir Dis* 1984; 130:565-70). However, in a subpopulation of patients where the cancer is diagnosed at a very early stage and the lung is surgically resectioned, there is a 5-year survival rate of 70-90%. (Flehinger, et al., "The effect of surgical treatment on survival from early lung cancer," *Chest;* 1992, 101:1013-1018; Melamed, et al., "Screening for early lung cancer: results of the Memorial Sloan-Kettering Study in New York," *Chest;* 1984 86:44-53). Therefore, research has focused on early detection of tumor markers before the cancer becomes clinically apparent and while the cancer is still localized and amenable to therapy.

The identification of antigens associated with lung cancer has stimulated considerable interest because of their use in screening, diagnosis, clinical management, and potential treatment of lung cancer. International workshops have attempted to classify the lung cancer antigens into 15 possible clusters that may define histologic origins. (Souhami, et al., "Antigens of lung cancer: results of the second international workshop on lung cancer antigens," *JNCI* 1991; 83:609-612). As of 1988, more than 200 monoclonal antibodies (MAb) have been reported to react with human lung tumors. (Radosevich, et al., "Monoclonal antibody assays for lung cancer," In:

Cancer Diagnosis in Vitro Using Monoclonal Antibodies. Edited by H. A. Kupchik. New York: Marcel Dekker, 1988).

MAbs for lung cancer were first developed to distinguish NSCLC from SCLC. (Mulshine, et al., "Monoclonal antibodies that distinguish nonsmall-cell from small-cell lung cancer," *J Immunol* 1983; 121:497-502). In most cases, the identity of the cell surface antigen with which a particular antibody reacts is not known, or has not been well characterized. (Scott, et al., "Early lung cancer detection using monoclonal antibodies," In: *Lung Cancer*. Edited by J.A. Roth, J.D. Cox, and W.K. Hong. Boston: Blackwell Scientific Publications, 1993).

MAbs have been used in the immunocytochemical staining of sputum samples to predict the progression of lung cancer. (Tockman, et al., "Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection," *J Clin Oncol* 1988; 6:1685-1693). In the study, two MAbs were utilized, 624H12 which binds a glycolipid antigen expressed in SCLC and 703D4 which is directed to a protein antigen of NSCLC. Of the sputum specimens from participants who progressed to lung cancer, two-thirds showed positive reactivity with either the SCLC or the NSCLC MAb. In contrast, of those that did not progress to lung cancer, 35 of 40 did not react with the SCLC or NSCLC Mab. This study suggests the need for the development of additional early detection targets to discover the onset of malignancy at the earliest possible stage.

Carcinoembryonic antigen (CEA) is a frequently studied tumor marker of cancer including lung cancer.

(Nutini, et al., "Serum NSE, CEA, CT, CA 15-3 levels in human lung cancer," *Int J Biol Markers* 1990; 5:198-202). Squamous cell carcinoma antigen is another established serum marker. (Margolis, et al., "Serum tumor markers in non-small cell lung cancer," *Cancer* 1994; 73:605-609.). Other serum antigens for lung cancer include antigens recognized by MAbs 5E8, 5C7, and 1F10, the combination of which distinguishes between patients with lung cancer from those without. (Schepart, et al., "Monoclonal antibody-mediated detection of lung cancer antigens in serum," *Am Rev Respir Dis* 1988; 138:1434-8) Furthermore, the combination of 5E8, 5C7 and 1F10 was more sensitive, specific and accurate for identifying NSCLC when compared to results from a combination of the CEA and squamous cell carcinoma antigen tests. (Margolis, et al., *Cancer* 1994; 73:605-609).

Serum CA 125, initially described as an ovarian cancer-associated antigen, has been investigated for its use as a prognostic factor in NSCLC. (Diez, et al., "Prognostic significance of serum CA 125 antigen assay in patients with non-small cell lung cancer," *Cancer* 1994; 73:136876). The study determined that the preoperative serum level of CA 125 antigen is inversely correlated with survival and tumor relapse in NSCLC.

Despite the numerous examples of MAb applications, none has yet emerged that has changed clinical practice. (Mulshine, et al., "Applications of monoclonal antibodies in the treatment of solid tumors," In: *Biologic Therapy of Cancer*. Edited by V.T. Devita, S. Hellman, and S.A. Rosenberg. Philadelphia: JB Lippincott, 1991, pp. 563-588). MAbs alone may not be the answer to early detection

because there has only been moderate success with immunologic reagents for paraffin-embedded tissue. Secondly, lung cancer may express features that cannot be differentiated by antibodies; for example, chromosomal deletions, gene amplification, or translocation and alteration in enzymatic activity.

After the gene to the MAb recognized surface antigen has been cloned, cytogenetic and molecular techniques may provide powerful tools for screening, diagnosis, management and ultimately treatment of lung cancer. An example of a lung cancer antigen that has been cloned is the adenocarcinoma-associated antigen. This antigen, recognized by KS1/4 MAb, is an epithelial malignancy/epithelial tissue glycoprotein from the human lung adenocarcinoma cell line UCLA-P3. (Strand, et al., "Molecular cloning and characterization of a human adenocarcinoma/epithelial cell surface antigen complementary DNA," *Cancer Res* 1989; 49:314-317). The antigen has been found on all adenocarcinoma cells tested and in various corresponding normal epithelial cells. Northern blot analysis indicated that transcription of the adenocarcinoma-associated antigen was detected in RNA isolated from normal colon but not in RNA isolated from normal lung, prostate, or liver. Therefore identification of adenocarcinoma-associated antigen in lung cells may prove to be diagnostic for adenocarcinoma.

The cloning of CEA and the nonspecific crossreacting antigen (NCA) has allowed the development of specific DNA probes which discriminate their expression in lung cancer at the mRNA level. (Hasegawa, et al., "Nonspecific crossreacting antigen (NCA) is a major member of the CEA-related gene family expressed in lung cancer," *Br J Cancer*

1993; 67:58-65). NCA is a component of the CEA gene family in lung cancer and is also recognized by anti-CEA antibodies, especially polyclonal antibodies. Because of the crossreactivity, investigations to analyze CEA and NCA separately in lung disease had been difficult. The use of DNA probes determined that lung cancer cells fall into three different types according to their CEA and/or NCA expression by Northern blot analysis. Specifically, lung cancers expressed both CEA and NCA mRNA, only NCA mRNA, or neither mRNA. CEA-related mRNA expression was always accompanied by NCA mRNA expression and there were no cases of CEA mRNA expression alone. The separate assessment of CEA and NCA expression in lung cancers may be important in determining the prognosis of lung cancers because the antigens have been described as cell-cell adhesion molecules and may play a role in cancer metastasis.

Another method to detect the presence of an antigen gene or its mRNA in specific cells or to localize an antigen gene to a specific locus on a chromosome is *in situ* hybridization. *In situ* hybridization uses nucleic acid probes that recognize either repetitive sequences on a chromosome or sequences along the whole chromosome length or chromosome segments. By tagging the probes with radioisotopes or color detection systems, chromosome regions can be identified within the cell. Investigations using *in situ* hybridization have demonstrated numerical chromosomal abnormalities in samples from human tumors, including bladder, neuroectodermal, breast, gastric and lung cancer tumors. (Kim, et al., "Interphase cytogenetics in paraffin sections of lung tumors by non-isotopic *in situ* hybridization. Mapping Genotype/phenotype heterogeneity," *Am J Pathol* 1993; 142:307-317).

Fluorescence *in situ* hybridization (FISH) allows cells to be stained so that genetic aberrations resulting in changes in gene copy number or structure can be quantitated by fluorescence microscopy. In this technique, a chemically labeled single-stranded nucleic acid probe homologous to the target nucleic acid sequence is annealed to denatured nucleic acid contained in target cells. The cells may be mounted on a microscope slide, in suspension or prepared from paraffin-embedded material. Treating the chemically modified probes with a fluorescent ligand makes the bound probe visible. FISH has been used for (1) detection of changes in gene copy number and gene structure; (2) detection of genetic changes, even in low frequency subpopulations; and (3) detection and measurement of the frequency of residual malignant cells. (Gray, et al., "Molecular cytogenetics in human cancer diagnosis," *Cancer* 1992; 69:1536-1542).

Other molecular markers for lung cancer include oncogenes and tumor suppressor genes. Dominant oncogenes are activated by mutation and lead to deregulated cellular growth. Such genes code for proteins that function as growth factors, growth factor receptors, signal transducing proteins and nuclear proteins involved in transcriptional regulation. Amplification, mutation, and translocations have been documented in many different cancer cells and have been shown to lead to gene activation or overexpression.

The *ras* family of oncogenes comprises a group of membrane associated GTP-binding proteins thought to be involved in signal transduction. Mutations within the *ras* oncogenes, resulting in sustained growth stimulation, have been identified in 15 to 30% of human NSCLC. (Birrer, et

al., "Application of molecular genetics to the early diagnosis and screening of lung cancer," *Cancer* 1992; 52suppl; 2658s-2664s). Patients with tumors containing *ras* mutations had decreased survival compared with patients whose tumors had no ras mutations. Polymerase chain reaction (PCR) amplification of *ras* genes can be analyzed to determine the presence of mutations by several methods: (a) differential hybridization of ^{32}P-labeled mutated oligonucleotides; (b) identification of new restriction enzyme sites created by the activating mutation; (c) single-strand conformational polymorphisms; and (d) nucleic acid sequencing. These methods combined with PCR technology could allow detection of an activated *ras* gene from sputum specimens.

Another family of dominant oncogenes, the *erb B* family, has been found to be abnormally expressed in lung cancer cells. This group codes for membrane-associated tyrosine kinase proteins and contains *erb B1*, the gene coding for the epidermal growth factor (EGF) receptor, and *erb B2* (also called *Her-2/neu*). The *erb B1* gene has been found to be amplified in NSCLC (up to 20% of squamous cell tumors), while the EGF receptor has been shown to be overexpressed in many NSCLC cells (approximately 90% of squamous cell tumors, 20 to 75% of adenocarcinomas, and rarely in large cell or undifferentiated tumors). (Birrer, et al., *Cancer* 1992: 52 suppl; 2658s-2664s). Amplification of the related oncogene *erb B2 (Her-2/neu)* occurs infrequently in lung cancer but is a negative prognostic factor in breast cancer. However, overexpression of the *erb B2* protein product, p185^{neu}, occurs in some NSCLC and may be related to poor prognosis. (Kern, et al., "p185^{neu} expression in human lung

adenocarcinomas predicts shortened survival," *Cancer Res* 1990; 50:5184-5191).

A third family of dominant oncogenes involved in lung cancer is the *myc* family. These genes encode nuclear phosphoproteins, which have potent effects on cell growth and which function as transcriptional regulators. Unlike *ras* genes, which are activated by point mutations in lung cancer cells, the *myc* genes are activated by overexpression of the cellular *myc* genes, either by gene amplification or by rearrangements, each ultimately leading to increased levels of *myc* protein. Amplification of the normal *myc* genes is seen frequently in SCLC and rarely in NSCLC.

The loss or inactivation of tumor suppressor genes may also be important steps in the pathway leading to invasive cancer. Tumor suppressor genes function normally to suppress cellular proliferation, and since they are recessive oncogenes, mutations or deletions must occur in both alleles of these genes before transformation occurs.

A phosphoprotein *p53*, which is encoded by a gene located on chromosome 17p, suppresses transformation in its wild-type state. While in its mutant state, *p53* acts as a dominant oncogene. *p53* functions in DNA binding and transcription activation. Mutations of *p53* have been found in many human cancers including colon, breast, brain and lung cancer cells. (Birrer, et al., *Cancer Res.*(suppl) 1992, 52:2658s-2664s). In NSCLC cell lines, *p53* mutations have been found at a rate of up to 74%. (Mitsudomi, et al., "*p53* gene mutations in non-small-cell lung cancer cell lines and their correlation with the

presence of *ras* mutations and clinical features," *Oncogene* 1992; 7:171-180).

Despite all of the advances made in the area of lung cancer, medical and surgical intervention has resulted in little change in the 5-year survival rate for lung cancer patients. Early detection holds the greatest hope for successful intervention. There remains a need for a practical method to diagnose lung cancer as close to its inception as possible. In order for early detection to be feasible, it is important that specific markers be found and their sequences elucidated.

A lung cancer marker antigen, specific for NSCLC, has now been found, sequenced, and cloned. The antigen is useful in methods for detection of non-small cell lung cancer and for potential production of antibodies and probes for treatment compositions.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 depicts the alignment of the amino acid sequence of HCAVIII with previously described carbonic anhydrases. Conserved amino acids are shown in bold.

SUMMARY OF THE INVENTION

The invention concerns a lung cancer antigen (HCAVIII) gene specific for non-small cell lung cancer.

In one embodiment, the invention relates to a substantially purified nucleic acid (SEQ ID NO:1) encoding the pre-protein sequence shown in SEQ ID NO:2.

In other embodiments, the invention relates to cDNAs which encode the mature form of the protein (SEQ ID NO:4), or a truncated form of the protein lacking the transmembrane domain (SEQ ID NO:13 and SEQ ID NO:15), or a protein in which one or more of the amino acids in the phosphorylation region have been altered to affect that function, an example of which is shown in SEQ ID NO:18.

In other embodiments, proteins encoded by the cDNA of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:12, SEQ ID NO:14, and SEQ ID NO:17 are provided.

In another aspect, the invention relates to a recombinant DNA clone for HCAVIII.

In further aspects of the invention, expression vectors for HCAVIII and modifications thereof are an object.

The invention further relates to methods of detecting lung cancer.

In one aspect an *in situ* hybridization technique is provided. In another aspect, a fluorescence *in situ* hybridization technique is provided. In a further aspect, an ELISA assay is provided. In another aspect, detection of carbonic anhydrase activity which correlates with lung cancer antigen is provided.

DETAILED DESCRIPTION OF THE INVENTION

The nucleic acid sequence coding for a cell surface protein (said protein hereinafter designated HCAVIII) which is highly specific for non-small cell lung cancer cells has now been obtained. This gene sequence will facilitate detection and treatment of the disease, which to date has often proven difficult.

The HCAVIII cDNA in the vector pLC56 has been sequenced and characterized including the entire coding region and substantially all of the upstream and downstream non-translated regions. The cDNA in pLC56 was sequenced on both strands from exonuclease III-generated deletions and subsequent subcloning into M13 vectors or directly from the cloning vectors using the di-deoxy method and a SEQUENASE ® Version 2.0 kit (U.S. Biochemicals, Cleveland, OH). Additional regions of DNA were subcloned as small restriction fragments into the same vectors for sequence analysis. Overlapping segments were ordered using MacVector Align software (Kodak/IBI Technologies, New Haven CT). SEQ ID NO:1 represents the cDNA encoding HCAVIII and a presumed signal peptide. SEQ ID NO:2 represents the signal peptide (amino acid residues -29 to -1) followed by the mature protein (amino acid residues 1 to 325). As predicted from the cDNA sequence in pLC56, a protein of about 354 amino acids is encoded with the predictive size of 39448 daltons. A hydrophilicity plot (MacVector software, Kodak/IBI Technologies) of this protein provided strong evidence of a leader peptide at the N-terminus and a membrane-spanning segment near the C-terminus. The membrane-spanning segment provides evidence that this protein is membrane bound, as also predicted by its positive selection with

panning methodology (See Watson, et al., *Recombinant DNA*, 2nd ed., pp. 115-116, 1992). The cleavage site of the signal as predicted by von Heijne (von Heijne, Gunnar, *Nucleic Acids Res* 1986; 14:4683-4690) is 29 amino acids down from the N-terminus methionine. SEQ ID NO:3 corresponds approximately to the coding region of the mature polypeptide. The subsequent "mature" protein is proposed to be 325 amino acids, initiating with serine, and of a calculated 36401 daltons and a pI of 6.42 (SEQ ID NO:4).

Homology searches against NCBI BlastN or BlastX version 1.3.12MP (National Center for Biotechnology Information, Bethesda, MD) provided evidence the gene and protein are novel, not previously identified in either database. (Altschul, et al., "Basic local alignment search tool," *J Mol Biol* 1990; 215:403-410). Additional searches against another database (Entrez, version 9) gave similar results.

The isolation of a second cDNA encoding HCAVIII permitted the identification of new sequences within the 5'-and 3'-prime untranslated regions of this gene. SEQ ID NO:5, a cDNA encoding HCAVIII and a portion of the 5' and 3' nontranslated regions, has substantial identity with SEQ ID NO:1 (positions 1-1104 of SEQ ID NO:1 are identical to positions 85-1188 of SEQ ID NO:5). The encoded protein is listed in SEQ ID NO:6 and is identical with SEQ ID NO:2. Homology searches of NCBI BlastN against SEQ ID NO:5 showed these gene sequences have not been previously identified. SEQ ID NO:7 represents additional cDNA sequences of the 3' nontranslated region of the HCAVIII gene located downstream from the sequences depicted in SEQ ID NO:5. Homology searches against the same data base

identified two clones with homology to SEQ ID NO:7. Both sequences are expressed sequence tags (EST), the first EST04899 (345 bp) and the second HUMGS04024 (466 bp).

Alignment searches indicate this protein shares common features with the seven human carbonic anhydrase proteins previously identified. However, as described below, certain structural features distinct to HCAVIII exist that may confer unique properties to this protein and a role in the transformation pathway to tumorgenicity. This group of enzymes catalyze the hydration of carbon dioxide

$$CO_2 + H_2O \approx HCO_3 + H^+$$

and in reverse the dehydration of HCO_3^-. This protein is identified as a carbonic anhydrase (CA) based on the conservation of amino acids at positions critical for the binding of Zn^{+2}, and the catalysis of CO_2, as well as numerous other conserved amino acids (see Fig. 1). The protein is 34 to 64 amino acids longer (at the C-terminus) than any previously reported carbonic anhydrase by virtue of the membrane-spanning region also found in HCAIV and an additional approximate 30 amino acids contained in the cytoplasmic side of the cell and apparently missing in other human CA isoforms. In addition, this intracellular domain contains a phosphorylation site recognized by protein kinase C and other kinases, as defined by the motif "Arg-Arg-Lys-Ser" (SEQ ID NO:8 and SEQ ID NO:9) (amino acid residues 1-4 in SEQ ID NO:9 and amino acid residues 299-302 in SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6).

Interestingly, this motif is found only in HCAVIII, and at a functionally significant site, i.e., within the cytosol. A surface cleft essential for enzymatic function present on other carbonic anhydrases is conserved for this protein, suggesting that this protein will also confer enzymatic activity. Five possible N-glycosylation sites are predicted by the primary amino acid sequence and the motif "Asn-Xaa-Ser (Thr)", beginning at amino acid residues -2, 51, 133, 151, and 202 in SEQ ID NO:2, respectively.

HCAVIII is expressed at a much higher level in a non-small cell lung cancer cell line (A549) than in normal lung tissue, other normal tissues, and other tumor cell lines which makes it useful in distinguishing this disease. This is clearly demonstrated in Table 1. Data for this table was obtained as follows. Total cellular RNA was isolated from the indicated actively growing cell lines as described by Chirgwin, et al., "Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease," *Biochemistry* 1979; 18:5294-5299. RNA samples were fractionated over a 1% agarose-formaldehyde gel and transferred to a nylon membrane (Qiagen, Chatsworth, CA) by capillary action. The hybridization probe was generated from a 1 kilobase pair BstXI restriction fragment isolated from pLC56, a plasmid harboring the HCAVIII gene in its initial isolation. This fragment was radiolabeled with ^{32}P using a PRIME-IT® Random Primer Labeling Kit obtained from Stratagene, La Jolla, CA. A membrane containing RNA derived from healthy human tissue was purchased from Clonetech Laboratories, Inc., Palo Alto, CA. RNA blots were hybridized in a standard cocktail containing ^{32}P-labeled probe at 42°C

overnight then exposed to X-ray film. The same blots were subsequently, upon removal of the probe, rehybridized with a second ^{32}P-labeled DNA from β-actin to serve as a positive control for integrity of the blotted RNA.

As shown in Table 1, normal lung tissue does not express the HCAVIII gene in detectable amounts. Other tumor cell lines fail to express, or express only in minor amounts, which will allow easy distinction of non-small cell carcinomas.

TABLE 1. NORTHERN BLOTS USING HCAVIII cDNA AGAINST NORMAL TISSUES AND TUMOR CELL LINES

TISSUE	mRNA (kB)	INTENSITY
NORMAL TISSUE		
heart	nd[1]	-----
brain	4.5	1X[2]
placenta	4.5	1X
lung	nd	-----
liver	nd	-----
skeletal muscle	nd	-----
kidney	4.5	100X
pancreas	4.5	10X
TUMOR CELL LINE		
A549 (lung carcinoma)	3.5	5000X
	5.4	50X
	8.0	25X
	9.0	25X
BT20 (breast carcinoma)	nd	-----
G361 (melanoma)	nd	-----
HT144 (melanoma)	nd	-----
U937 (histiocytic lymphoma)	nd	-----
KG-1 (myelogenous leukemia)	nd	-----

[1] nd = none detected

[2] 1X = at limit of detection

In one embodiment of the invention, probes are made corresponding to sequences of the cDNA shown in SEQ ID NO:3, which are complimentary to the mRNA for HCAVIII. These probes can be radioactively or non-radioactively labeled in a number of ways well known to the art. The probes can be made of various lengths. Such factors as stringency and GC content may influence the desired probe length for particular applications. The probes correspond to a length of 10-986 nucleotides from SEQ ID NO:3. The labeled probes can then be bound to detect the presence or absence of mRNA encoding the HCAVIII in biopsy material through *in situ* hybridization. The mRNA is expected to be associated with the presence of non-small cell tumors and to be a marker for the precancerous condition as well.

In situ hybridization provides a specificity to the target tissue that is not obtainable in Northern, PCR or other probe-driven technologies. *In situ* hybridization permits localization of signal in mixed-tissue specimens commonly found in most tumors and is compatible with many histologic staining procedures. This technique is comprised of three basic components: first is the preparation of the tissue sample provided by the pathologist to permit successful hybridization to the probe. Second is the preparation of the hybridization probe, typically a RNA complementary to the mRNA of the gene of interest (i.e., antisense RNA). RNA probes are preferred over DNA probes for *in situ* hybridizations mainly because background hybridization of the probe to irrelevant nucleic acids or nonspecific attachment to cell debris or subcellular organelles can be eliminated with RNAse treatment post-hybridization. Third is the hybridization and post-hybridization detection. Typically

the RNA transcript probe has been radiolabeled by the incorporation of ^{32}P or ^{35}S nucleotides to permit subsequent detection of the probed specimen by autoradiography or quantitation of silver grains following treatment with autoradiographic emulsion. Nonradioactive detection systems have also been developed. In one example, biotinylated nucleotides can be substituted for the radioactive nucleotide in the RNA probe preparation, permitting visualization of the probed sample by immunocytochemistry-derived techniques. Example 1 describes *in situ* hybridization procedures using RNA probes derived from the HCAVIII gene. Example 2 provides exemplary fluorescent *in situ* (FISH) hybridization procedures.

The cDNA for HCAVIII (SEQ ID NO:3) is currently in an expression vector which is be used to generate the protein in *E. coli*. This expression system described in Example 3 produces HCAVIII to be used as an antigen for the generation of antibodies (Example 4) for use in an ELISA assay to detect shed HCAVIII in body fluids as described in Example 5. The methods for production of antibodies and ELISA type assays are well known in the art. Exemplary methods and components of these procedures have been chosen and developed and are described in Examples 4 and 5.

The expression and purification of foreign proteins in *E. coli* is often problematic. On occasion, the protein is expressed at high levels but is deposited within the cell as an insoluble, denatured form termed an inclusion body. These bodies are often observed when the foreign protein contains a hydrophobic domain, such as found in the membrane spanning segment of HCAVIII. Through

recombinant DNA technology, the DNA sequences encoding the membrane spanning segment of HCAVIII are deleted. The protein expressed in *E. coli* from this engineered plasmid is now in a soluble and native form within the cell, permitting a rapid and less harsh purification. In addition, the ELISA test to measure HCAVIII shed into body fluids as described in Example 5 relies on the recombinant protein produced from *E. coli*. Typically, the shed antigen is a membrane-bound receptor that was released from the membrane spanning segment anchoring it to the cell. Consequently, the recombinant HCAVIII engineered to remove the membrane spanning segment is a more accurate representation of the putative HCAVIII shed antigen found in specimens and may prove to be the preferred antigen for polyclonal antisera and monoclonal antibody production as described for the development of an ELISA test.

To produce the engineered plasmid, a first plasmid is constructed by cleaving pLC56 with the restriction enzyme Tth111 I, followed by treatment with T_4-DNA polymerase and dGTP, dATP, dTTP and dCTP, and finally with alkaline phosphatase to remove 5'-terminal phosphates. The DNA sample is then purified by phenol/chloroform extraction and ethanol precipitation. The sample is digested with the restriction endonuclease BspE1, then the fragments are resolved by agarose gel electrophoresis to permit the isolation of a 267 base pair fragment. A second plasmid described previously for expression of the HCAVIII mature protein (SEQ ID NO:4), is cleaved with EcoRI and BspE1 followed by alkaline phosphatase treatment and purification by phenol/chloroform extraction and ethanol precipitation. Two oligonucleotides are synthesized, being 5'-TGAGTCGACG (SEQ ID NO:10) and 5'-AATTCGTCGACTCA

(SEQ ID NO:11), that complement each other and upon annealing, provide a termination codon (TGA) and sequence complementary to EcoRI cleaved DNA. Finally, the two oligonucleotides, the 267 base pair fragment, and the BspEI/EcoRI cleaved plasmid will be combined in a ligation reaction, and the resultant plasmid which contains the truncated DNA sequence (SEQ ID NO:12) is used to transform competent *E. coli*. Upon expression in *E. coli*, the resulting truncated protein (SEQ ID NO:13) is 271 amino acids as determined by SDS polyacrylamide electrophoresis and of a size consistent with other HCA's but lacking the membrane spanning segment and the intracellular domain. A second plasmid encoding a HCAVIII truncated protein (SEQ ID NO:14) lacking the membrane spanning segment and intracellular domain was created as described above, except that restriction enzyme Ple I was substituted for Tth111 I, resulting in a gel purified DNA fragment of 276 base pairs. Upon expression in E.coli, the resulting protein is now 274 amino acids (SEQ ID NO:15).

An understanding of protein phosphorylation and its role in the mechanism of cell transformation has been actively pursued, most notably with tyrosine phosphorylation and oncogene activation. The role of serine/threonine protein phosphorylation by a variety of protein kinases including protein kinase C has been studied extensively with respect to signal transduction, but its role in oncogenesis is less clear. To provide a valuable tool to be used in the study of the role of HCAVIII serine phosphorylation in oncogenesis, an altered cDNA can be prepared to code for an altered protein. Changes to amino acids other than "Gly" may be realized by alterations to the oligonucleotide sequence (SEQ ID NO:16)

used to encode the selected residue. Other modifications to alter the serine phosphorylation site would utilize the described technology to modify either both "Arg" residues located within SEQ ID NO:9 or amino acid residues 299 and 300 of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6. Since "Arg" residues contain a net positive charge, the substituted amino acids would preferably be "Lys" or "His," also positively charged amino acids. An exemplary plasmid is produced in which the "Ser" codon (amino acid residue 4 of SEQ ID NO:9; amino acid residue 302 in SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6), is converted to a "Gly" codon using an *in vitro* mutagenesis technique described in Example 3 and previously recited in Kunkel, Thomas, "Rapid and efficient site-specific mutagenesis without phenotypic selection," *Proc Natl Acad Sci USA* 1985; 82:488-492, and the oligonucleotide 5'-CTTTTTTGATACCCTTCCTTCTGAA (SEQ ID NO:16) (located in SEQ ID NO:1 at the base pairs 1010-1034 with 1022 as the mutagenized base pair). The DNA sequences containing the HCAVIII gene engineered for production of the mature protein and mutagenized codon is released from the mutagenesis vector by BamHI and EcoRI restriction endonucleases and ligated into pGEX4T1 cleaved with the same enzymes, and the resultant plasmid is used to transform competent *E. coli*. The codon mutagenesis is confirmed by DNA sequence analysis, and the protein is expressed and purified from *E. coli* as described in Example 3. The DNA sequence of the altered plasmid as shown in SEQ ID NO:17 differs from the gene encoding the mature protein (SEQ ID NO:3) in that the nucleotide 1022 is changed from "A" to "G", and the protein sequence (SEQ ID NO:18) expressed by the altered plasmid is identical to

the mature protein (SEQ ID NO:4) except that amino acid residue 302 is changed from "Ser" to "Gly."

Another way to detect the presence of increased HCAVIII could be to assay for levels of carbonic anhydrase activity in biopsy materials as described in Example 6. This should be a useful test as HCAVIII, although it is an immunologically unique molecule, contains small but distinct regions which are conserved between previously reported carbonic anhydrase proteins.

In another embodiment of the invention, primers are made complimentary to the HCAVIII cDNA (SEQ ID NO:3) for detecting expression of the gene. PCR amplification of cDNA from lung biopsy cells would indicate the presence of the same non-small cell lung carcinoma.

Due to the non-small cell lung cancer specificity of HCAVIII and the gene encoding the protein, antibodies specific for HCAVIII would also exhibit non-small cell lung cancer specificity which can be employed for diagnostic detection of HCAVIII in body fluids such as serum or urine or HCAVIII containing cells. Targeting of cancer therapeutic drugs to HCAVIII containing cells can also be developed using HCAVIII specific antibodies. The genetic expression of the gene encoding HCAVIII could be modulated by drugs or anti-sense technology resulting in an alteration of the cancer state of the HCAVIII containing cells.

Example 1

In Situ Hybridization using RNA Probes Derived from the HCAVIII Gene

Tissue samples are treated with 4% paraformaldehyde (or equivalent fixative), dehydrated in sequential ethanol solutions of increasing concentrations (e.g., 70%, 95% and 100%) with a final xylene incubation (see *Current Protocols in Molecular Biology*, pp. 14.01-14.3 and *Immunocytochemistry II:IBRO Handbook Series: Methods in the Neurosciences* Vol 14; pp 281-300, incorporated herein by reference). The tissue is embedded in molten paraffin, molded in a casting block and can be stored at room temperature. Tissue slices, typically 8 µm thick, are prepared with a microtome, dried onto gelatin-treated glass slides and stored at -20°C.

DNA sequences from the HCAVIII gene (SEQ ID NO:3) are subcloned into a plasmid engineered for production of RNA probes. In this example, a 776 bp DNA fragment is released from a pLC56 plasmid following BamHI/AccI digestion, where the BamHI site has been created by in vitro mutagenesis (see *E. coli* expression below). This fragment is ligated into pGEM-2 (Promega Biotec, Madison, WI) that was cleaved with BamHI and AccI and transformed into competent *E. coli*. This constructed plasmid contains the T7 RNA polymerase promoter downstream of the AccI restriction site and hence can drive transcription of the antisense HCAVIII sequences defined by the BamHI/AccI fragment. Following linearization of the subsequent plasmid with BamHI, an in vitro transcription reaction composed of transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 1 U/ul ribonuclease inhibitor), linearized plasmid, 10 mM GTP, 10 mM ATP, 10 mM CTP, 100 µCi of (^{35}S)UTP, and T7 RNA polymerase is incubated at 37°C. Multiple RNA copies of the gene are produced that then are

used as a hybridization probe. The reaction is terminated by the addition of DNAase, and the synthesized RNA is recovered from unincorporated nucleotides by phenol/chloroform extraction and sequential ethanol precipitations in the presence of 2.5 M ammonium acetate.

The slides containing fixed, sectioned tissues are rehydrated in decreasing concentration of ethanol (100%, 70% and 50%), followed by sequential treatments with 0.2 N HCl, 2X SSC (where 20X SSC is 3 M NaCl and 0.3 M sodium citrate) at 70°C to deparaffinate the sample , phosphate buffered saline (PBS), fixation in 4% paraformaldehyde and PBS wash. The slides are blocked to prevent nonspecific binding by the sequential additions of PBS/10mM dithiothreitol (45°C), 10 mM dithiothreitol/0.19% iodoacetamide/0.12% N-ethylmaleimide and PBS wash. The slides are equilibrated in 0.1M triethylamine, pH 8.0, followed by treatment in 0.1M triethylamine/0.25% acetic anhydride and 0.1 M triethylamine/0.5% acetic anhydride and washed in 2X SSC. The slides are then dehydrated in increasing concentrations of ethanol (50%, 70% and 100%) and stored at -80°C.

A hybridization mix is prepared by combining 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1X Denhardt's solution (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (BSA)), 500 µg/ml yeast tRNA, 500 µg/ml poly(A), 50 mM dithiothreitol, 10% polyethyleneglycol 6000 and the ^{35}S-labeled RNA probe. This solution is placed on the fixed, blocked tissue slides which are then incubated at 45°C in a moist chamber for 0.5 to 3 hours. The slides are washed to remove unbound probe in 50% formamide, 2X SSC, 20 mM 2-mercaptoethanol (55°C), followed by 50% formamide, 2X SSC,

20 mM 2-mercaptoethanol and 0.5% Triton-X 100 (50°C) and finally in 2X SSC/20 mM 2-mercaptoethanol (room temperature). The slides are treated with 10 mM Tris-HCl, pH 8.0/0.3 M NaCl/40 µg/ml RNase A/2 µg/ml RNAse T1 (37°C) to reduce levels of unbound RNA probe. Following RNAse treatment, the slides are washed in formamide/SSC buffers at 50°C, room temperature and then dehydrated in increasing ethanol concentrations containing 0.3 M ammonium acetate, and one final 100% ethanol wash. The slides are then exposed to X-ray film followed by emulsion autoradiography to detect silver grains.

Test tissue samples are compared to matched controls derived from normal lung tissue. Evidence of elevated transcription of the HCAVIII gene in test tissue compared to normal tissue, as determined by autoradiography (X-ray film) or alternatively by the quantitation of silver grains following emulsion autoradiography would provide evidence of a positive diagnosis for lung cancer.

Example 2

Fluorescent *In Situ* Hybridization (FISH) Using DNA Probes Derived from the HCAVIII Gene

A genomic clone to the HCAVIII gene (SEQ ID NO:1) is isolated using a PCR primer pair which have been identified from the pLC56 cDNA sequence. This primer pair is located in putative exon 6 of the pLC56 gene, and they are identified as Probe Exon 6A (5'-ACATTGAAGAGCTGCTTCCGG-3'; SEQ ID NO:19) and Probe Exon 6B (5'-AATTTGCACGGGGTTTCGG-3'; SEQ ID NO:20). The genomic clone of HCAVIII is then identified as a PCR product of about 119 bp using this primer pair from the designated genomic clone. This result is confirmed by Southern blotting and

DNA sequence analysis. A sequence of 1363 bp derived from the HCAVIII genomic clone is reported in SEQ ID NO:21. This sequence is located directly before the HCAVIII cDNA and constitutes the putative promoter of this gene and likely contains transcription regulatory elements directly implicated in HCAVIII expression.

The DNA probe comprising the genomic clone of HCAVIII plus flanking sequences is labeled in a random primer reaction with digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN) by combining the DNA with dNTP(-TTP, final 0.05 mM), digoxigenin-11-dUTP/dTTP (0.0125 mM and 0.0375 mM, final), 10 mM 2-mercaptoethanol, 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 20 U of DNA polymerase I and 1 ng/ml DNAase. The reaction is incubated at 15°C for two hours, and then terminated by adding EDTA to a final concentration of 10 mM. The labeled DNA probe is further purified by gel filtration chromatography. It is apparent to those skilled in the art that other suitable substrates such as biotin-11-dUTP can be substituted for digoxigenin-11-dUTP in the procedure above.

A hybridization mix is prepared by combining 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1X Denhardt's solution (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 500 μg/ml yeast tRNA, 500 μg/ml poly(A), 50 mM dithiothreitol, 10% polyethyleneglycol 6000, and the labeled DNA probe.

Single cell suspensions of tissue biopsy material or normal tissue are fixed in methanol/glacial acetic acid (3:1 vol/vol) and dropped onto microscope slides. (Aanastasi, et al., "Detection of Trisomy 12 in chronic lymphocytic leukemia by fluorescence in situ hybridization

to interphase cells: a simple and sensitive method," *Blood* 1992; 77:2456-2462). After the slides are heated for 1-2 hours at 60°C, the hybridization mix is applied to the slides which are then incubated at 45°C in a moist chamber for 0.5-3 hours. After incubation, the slides are washed three times with a solution comprising 50% formamide and 2X SSC at 42°C, washed twice in 2X SSC at 42°C, and finally washed in 4X SSC at room temperature. The slide is blocked with a solution of 4X SSC and 1% BSA, and then washed with a solution of 4X SSC and 1% Triton X-100.

The hybridized digoxigenin-labeled probe is detected by adding a mixture of sheep anti-digoxigenin antibody (Boehringer Mannheim) diluted in 0.1 M sodium phosphate, pH 8.0, 5% nonfat dry milk, and 0.02% sodium azide, followed by the addition of fluorescein-conjugated rabbit anti-sheep IG for detection. The slides are then washed in PBS, mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA), and viewed by fluorescent microscopy.

Hybridization signals are enumerated in tumor derived tissue and then compared to normal tissue. Normal tissue displays two distinct hybridization signal characteristics of a diploid state. Enumeration over the rate of two hybridization signals/cell is considered significant.

Example 3

Expression of HCAVIII

Expression of foreign proteins is often performed in *E. coli* when an immunogen or large amounts of protein are desired, as in the development of a diagnostic kit. A preferred system for *E. coli* expression has been described (Smith, et al., "Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione-

s-transferase," *Gene* 1988; 67:31-40) whereby glutathione transferase is expressed with amino acids representing the cloned protein of interest attached to the carboxyl-terminus. The fusion protein can then be purified via affinity chromatography and the protein of interest fused to glutathione transferase released by digestion with the protease thrombin or alternatively the fusion protein is released intact from the affinity column by competing levels of free glutathione.

To express the HCAVIII protein (SEQ ID NO:4) of this invention in *E. coli* using the above described technology, an expression plasmid was produced fused to the glutathione transferase gene in frame with the HCAVIII gene (SEQ ID NO:1) to produce a fusion protein. The fusion gene/expression plasmid was assembled from nucleic acids derived from the following sources. First, the expression plasmid pGEX4T1 (Pharmacia, Piscataway, NJ) was cleaved in the polycloning region with the restriction endonucleases BamHI and EcoRI to permit insertion of the HCAVIII gene. Second, an oligonucleotide was synthesized, being 5'-GTCCACTTGGATCCGTTCACTGG-3' (SEQ ID NO:22). Using the in vitro mutagenesis procedure described by Kunkel (*Proc Natl Acad Sci USA* 1985; 82:488-492) and the above oligonucleotide, a BamHI restriction site was created without altering the amino acid codons of the original protein. In addition the created BamHI site was situated in correct reading frame and proximity to the predicted cleavage site separating the signal peptide from the mature protein. The DNA sequences encoding the mature protein were released from the mutagenesis vector as a BamHI/EcoRI fragment, where the EcoRI site originates from a polycloning region of the DNA sequencing vector pUC19

found downstream of the HCAVIII gene. The DNA fragments described above comprised of pGEX4T-1 cleaved at BamHI and EcoRI and the HCAVIII gene released as a BamHI/EcoRI fragment was combined in a mixture composed of 1X T_4 ligase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, 50 µg/ml BSA, final pH 7.5) and T_4 DNA ligase (New England Biolabs, Beverly, MA). The ligated DNA was used to transform a suitable strain of *E. coli* such as XL-1 Blue (Stratagene). The recovered plasmid is sequenced to confirm the expected DNA sequence. Protein expression is induced in *E. coli* with the chemical isopropyl β-thiogalactoside, and the fusion protein is released by cell lysis, followed by denaturation and resolubilization of the fusion protein with 8 M urea/ 20 mM Tris.Cl (pH 8.5)/10 mM dithiothreitol, dialysis and protein renaturation, and finally binding to an affinity column composed of glutathione-agarose (Sigma, St. Louis, MO) and cleavage with thrombin to release the HCAVIII protein. The resulting protein is suitable as an immunogen for polyclonal or monoclonal antibody production and for usage in an ELISA kit as a internal standard and positive control. Carbonic anhydrase enzyme activity (as described in Example 6) was measured for E.coli-derived HCAVIII and HCAVIII-truncated form (SEQ ID NO:15) and compared to commercially obtained human carbonic anhydrase II (Sigma, St. Louis, Mo.). The activity, as reported in Enzyme Unit (U)/mg, for human carbonic anhydrase II was 3571 U/mg, for HCAVIII was 274 U/mg and HCAVIII truncated form was 2632 U/mg. These results indicated an enzymatically active and renaturable HCAVIII derived from *E.coli* of comparable enzymatic activity to human carbonic anhydrase II was obtained.

The length of the resulting protein can be varied by altering the length of SEQ ID NO:1 prior to insertion into the expression plasmid, or by cleavage of amino acids from the protein resulting in the above example. Structure/ function studies of other HCA's suggest modifications (as defined by deletions at the N-terminal and C-terminal) more extensive than disclosed in SEQ ID NO:12 would still permit the production and use of a protein as an immunogen or standard, these deletions being a protein defined by about amino acid residue 3 to amino acid residue 259 in SEQ ID NO:12. Using existing technology one could synthesize a peptide of approximately 10 to 40 amino acids in length that comprises a structural domain of HCAVIII. This synthesized peptide, coupled to a carrier protein, could be used for generating polyclonal antisera specific for native HCAVIII.

Example 4

Production of Antibodies to HCAVIII

The production of polyclonal antisera is described in great detail in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, 1988 incorporated herein by reference. The HCAVIII protein (SEQ ID NO:4) in the presence of an adjuvant is injected into rabbits with a series of booster shots as a prescribed schedule optimal for high titers of antibody in serum. A total of seven biweekly bleeds were obtained from two rabbits immunized with HCAVIII truncated protein (SEQ ID NO:15). The resulting anti-HCAVIII serum titer was compared to preimmune sera of the same rabbits and determined to be 1000 to 2000-fold greater, hence suitable as a reagent for indirect ELISA (Example 5). Rabbit

antibody was partially purified by precipitation with ammonium sulfate (50%, final) followed by dialysis and fractionation by preparative DEAE-HPLC.

An extensive description for producing monoclonal antibodies derived from the spleen B cells of an immunized mouse and a immortalized myeloma cell is found in the above reference for polyclonal antisera production. Mice are immunized with either the purified HCAVIII protein or a glutathione/HCAVIII fusion protein. Following cell fusion, selection for hybrid cells and subcloning, hybridomas are screened for a positive antibody against whole A549 cells or purified HCAVIII protein using an indirect ELISA assay as described for the ELISA kit (see Example 5).

Example 5

ELISA Assay of Shed HCAVIII

An indirect ELISA screening assay for HCAVIII protein (SEQ ID NO:4) has been designed to detect and monitor the HCAVIII protein in body fluids including but not limited to serum and other biological fluids such as sputum or bronchial effluxion at effective levels necessary for sensitive but accurate determinations. It is intended to aid in the early diagnosis of non-small cell lung cancer, for which there currently is no effective treatment. An early-detection, accurate, non-invasive assay for non-small cell lung cancer would be of great benefit in the management of this disease.

The immunochemicals used in this procedure were rabbit anti-human HCAVIII antibody (purified IgG, IgM) produced according to the procedure given in Example 4, mouse anti-human HCAVIII (monoclonal) also produced

according to the procedure given in Example 4, and goat anti-Rabbit IgG/peroxidase conjugate. The HCAVIII protein standard and internal positive control were produced as described in Example 3 for expression in *E. coli*.

Substrate components include 1 M H_2SO_4 stored at room temperature and 3',5,5'-tetramethylbenzidine (TMB) (Sigma Chemical Co.) used as a peroxidase substrate and stored at room temperature in the dark to prevent exposure to light.

Several buffers, diluents, and blocking agents were used in the procedure. Note that no sodium azide preservative was used in any of the buffers. This was done to avoid any possible interference from the azide with the peroxidase conjugate.

Phosphate buffered saline (PBS) was prepared by adding 32.0 g sodium chloride, 0.8 g potassium phosphate, monobasic, 0.8 g potassium chloride, and 4.6 g sodium phosphate, dibasic, anhydrous, to 3.2 L deionized water and mixing to dissolve. After bringing the solution to 4 L with deionized water and mixing, the pH was about 7.2. The buffer can be stored at 4°C for a maximum of 3 weeks.

Two bovine serum albumin solutions (BSA) were utilized as diluents. A 1% BSA solution in PBS, utilized as the second antibody/conjugate diluent, was prepared by adding 1 g BSA (bovine albumin, Fraction V, Sigma Chemical Co.) to 80 ml of PBS, allowing it to stand as it slowly goes into solution, adding PBS to a final volume of 100 ml, and then mixing. This diluent can be stored at 4°C for a maximum of 2 weeks; however if the solution becomes turbid, it should be discarded. As a diluent for the standards and samples, a 0.025% BSA solution in PBS was prepared fresh for each assay by diluting the 1% BSA diluent with PBS 1:40 (vol/vol).

A borate blocking buffer (0.17 M H_3BO_3, 0.12 M NaCl, 0.05% Tween 20, 1mM EDTA and 0.25% BSA was also used.

The substrate buffer was phosphate-citrate/sodium per borate (Sigma, St. Louis, Mo.).

All assays were performed in Immulon IV plates (Dynatech, Chantilly, VA #011-010-6301). The assay plates were coated with a monoclonal antibody against HCAVIII by adding 50 ul of a 10 ug/ml solution of antibody in PBS to each well of Immulon IV plates. The plates were covered and incubated overnight at room temperature. The antibody solution was removed and the wells rinsed three times with deionized water. Three-hundred microliters (300 ul) of the borate blocking buffer was added to each well and incubated at room temperature for thirty minutes. The buffer was removed, the wells rinsed three times with deionized water, and the plates air dried. The plates were then wrapped and stored at 4°C.

The standard E.coli-derived HCAVIII truncated protein (SEQ ID NO:15), was diluted to 32 ng/ml in PBS/0.025% BSA and two-fold serial dilutions were made in same. The samples were also diluted in PBS/0.025% BSA and 50 ul of standard or sample was applied to each well. The plates were incubated overnight, covered, at room temperature.

The standard and sample solutions were removed from the wells and the wells were rinsed three times with deionized water. Three-hundred microliters (300 ul) borate blocking buffer was added to each well and incubated at room temperature for thirty minutes. The plates were rinsed again with deionized water and tapped (inverted) on paper towels to remove excess water. The second antibody rabbit antisera to HCAVIII truncated protein (SEQ ID NO:15), was diluted to 1 ug/ml in PBS/1%

BSA and 50 ul was added to each well. The plates were covered and incubated at room temperature two hours.

The antibody solution was removed from the wells which were then rinsed with deionized water three times. They were then blocked for ten minutes at room temperature with borate blocking buffer, rinsed again with deionied water three times, and tapped on paper towels. The antibody conjugate, goat F(ab')2 x rabbit IgG & IgL-HPRO (Tago, Camarillo, CA.) was diluted 1:16,000 in PBS/1%BSA and 50 ul was added to each well. The plates were covered and incubated at room temperature two hours.

The antibody conjugate solution was removed from the wells and they were rinsed with deionized water three times, blocked with three-hundred ul borate buffer at room temperature then minutes, rinsed three times with deionized water, and tapped on paper towels. The substrate was prepared no more than fifteen minues before use by dissolving one capsule of phosphate-citrate/sodium perborate (Signma, St. Louis, Mo.) in 100 ml water. For each plate, one tablet of TMB was added to 10 ml of the phosphate-citrate/sodium perborate buffer and syringe filtered. One-hundred ul was added to each well and the plates were covered and incubated at room temperature in the dark for one hour. The reaction was stopped by adding 50 ul of 1M H_2SO_4 to each well. The plates were read on a Molecular Devices microplate reader at 450nm. Under these conditions, a linear response was obtained from 0.5 to 32 ng/ml using HCAVIII truncated protein as a standard, with the assay sensitivity at 0.5 ng/ml. No cross-reaction was observed against HCAII, an abundant carbonic anhydrase in human serum.

Example 6

Carbonic Anhydrase (CA) Activity of Biopsy Tissue

Ice cold solutions of ITB (20 mM imidazole, 5 mM Tris, and 0.4 mM para-nitrophenol, pH 9.4-9.9) and Buffer A (25 mM triethanolamine, 59 mM H_2SO_4, and 1 mM benzamidine HCl) are prepared.

A homogenate is prepared by scraping with a cell scraper into 1-2 ml of Buffer A a monolayer of tissue cells cultured from a tissue sample taken from a biopsy. A portion of the sample is then boiled to inactivate CA.

A tube is placed in an ice water bath. For the macroassay, a 10 x 75 mm glass tubes and rubber stopper with 16 gauge and 18 gauge needle ports is used; for the microassay, a 6 x 50 mm glass tubes and rubber stopper with 18 gauge needle port and 20 gauge needle with attached PE90 tubing. The sample is added and along with ice cold water to a final volume of 500 µl for macroassay or 50 µl for microassay. 500 µl (macro) or 50 µl (micro) ice cold water is used for a water control. 10 µl antifoam (A. H. Thomas, Philadelphia, PA) is added to the tube which is then incubated in ice water for 0.5 to 3 minutes.

The tube is capped with a stopper and CO_2 at 150 ml/min (macro) or 100 ml/min (micro) is bubbled through the smaller needle port for 30 sec.

50 µl (macro) or 50 µl (micro) of the ITB solution is rapidly added through the larger needle port with a cold Hamilton syringe. The sample becomes yellow.

Using a timer or stopwatch, the time at which the solution in the tube becomes colorless is measured and recorded. The tube may be momentarily removed from the bath and held in front of a white background to determine

the color change. Comparison to a previously acidified sample may be used.

The procedure is repeated with the boiled sample. The volume of sample that corresponds to approximately one enzyme unit is determined using the formula below.

Volume (1EU) = V_{EU} = volume used x log2/log (boiled time/activated time) One enzyme unit is the activity that halves the boiled control time.

The assay is repeated 1-3 times with the sample and boiled sample, using the adjusted volume of sample.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Lung Cancer Marker

(iii) NUMBER OF SEQUENCES: 22

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 32..1093

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 119..1093

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1013..1024
(D) OTHER INFORMATION: /note= "phosphorylation site recognized by protein kinase C and other kina..."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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                                   -29             -25

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Ala Ala Ala Val Leu Leu Leu Val Ile Leu Lys Glu Gln Pro Ser Ser
       -20                 -15                 -10

CCG GCC CCA GTG AAC GGT TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG        148
Pro Ala Pro Val Asn Gly Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly
    -5                   1                   5                  10

GAG AAT AGC TGG TCC AAG AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG        196
Glu Asn Ser Trp Ser Lys Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln
                15                  20                  25

TCC CCC ATA GAC CTG CAC AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC        244
Ser Pro Ile Asp Leu His Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu
            30                  35                  40

ACG CCC CTC GAG TTC CAA GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT        292
Thr Pro Leu Glu Phe Gln Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe
        45                  50                  55

CTC CTG ACC AAC AAT GGC CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC        340
Leu Leu Thr Asn Asn Gly His Ser Val Lys Leu Asn Leu Pro Ser Asp
    60                  65                  70

ATG CAC ATC CAG GGC CTC CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC        388
Met His Ile Gln Gly Leu Gln Ser Arg Tyr Ser Ala Thr Gln Leu His
75                  80                  85                  90

CTG CAC TGG GGG AAC CCG AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC        436
Leu His Trp Gly Asn Pro Asn Asp Pro His Gly Ser Glu His Thr Val
                95                 100                 105

AGC GGA CAG CAC TTC GCC GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA        484
Ser Gly Gln His Phe Ala Ala Glu Leu His Ile Val His Tyr Asn Ser
            110                 115                 120
```

```
GAC CTT TAT CCT GAC GCC AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC        532
Asp Leu Tyr Pro Asp Ala Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu
        125                 130                 135

GCT GTC CTG GCT GTT CTC ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT        580
Ala Val Leu Ala Val Leu Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr
    140                 145                 150

GAC AAG ATC TTC AGT CAC CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA        628
Asp Lys Ile Phe Ser His Leu Gln His Val Lys Tyr Lys Gly Gln Glu
155                 160                 165                 170

GCA TTC GTC CCG GGA TTC AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC        676
Ala Phe Val Pro Gly Phe Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr
                175                 180                 185

GCT GAA TAT TAC CGC TAC CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC        724
Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn
            190                 195                 200

CCC ACT GTG CTC TGG ACA GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG        772
Pro Thr Val Leu Trp Thr Val Phe Arg Asn Pro Val Gln Ile Ser Gln
        205                 210                 215

GAG CAG CTG CTG GCT TTG GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC        820
Glu Gln Leu Leu Ala Leu Glu Thr Ala Leu Tyr Cys Thr His Met Asp
    220                 225                 230

GAC CCT TCC CCC AGA GAA ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG        868
Asp Pro Ser Pro Arg Glu Met Ile Asn Asn Phe Arg Gln Val Gln Lys
235                 240                 245                 250

TTC GAT GAG AGG CTG GTA TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT        916
Phe Asp Glu Arg Leu Val Tyr Thr Ser Phe Ser Gln Val Gln Val Cys
                255                 260                 265

ACT GCG GCA GGA CTG AGT CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT        964
Thr Ala Ala Gly Leu Ser Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala
            270                 275                 280

GGC ATT CTT GGC ATC TGT ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC       1012
Gly Ile Leu Gly Ile Cys Ile Val Val Val Val Ser Ile Trp Leu Phe
        285                 290                 295

AGA AGG AAG AGT ATC AAA AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG       1060
Arg Arg Lys Ser Ile Lys Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys
    300                 305                 310

CCA GCC ACC AAG ATG GAG ACT GAG GCC CAC GCT TGAGGTCCCC G              1104
Pro Ala Thr Lys Met Glu Thr Glu Ala His Ala
315                 320                 325
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Arg Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile
-29              -25                 -20                 -15

Leu Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp
           -10                 -5                  1

Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro
      5                  10                  15

Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile
 20                  25                  30                  35

Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn
                40                  45                  50

Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val
            55                  60                  65

Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg
        70                  75                  80

Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro
     85                  90                  95

His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu
100                 105                 110                 115

His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala
                120                 125                 130

Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met
            135                 140                 145

Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His
        150                 155                 160

Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu
    165                 170                 175

Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser
180                 185                 190                 195

Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg
                200                 205                 210

Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala
            215                 220                 225

Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn
        230                 235                 240
```

```
Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser
    245                 250                 255

Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile
260                 265                 270                 275

Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val
                280                 285                 290

Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp
            295                 300                 305

Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala
        310                 315                 320

His Ala
    325
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 986 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..975

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 895..906
(D) OTHER INFORMATION: /note= "phosphorylation site recognized by protein C kinase and other kina..."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG      48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
  1               5                  10                  15

AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC      96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
            20                  25                  30

AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA     144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
        35                  40                  45

GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC     192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
    50                  55                  60
```

```
CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC        240
His Ser Val Lys Leu Asn Leu Pro S r Asp Met His Ile Gln Gly Leu
 65                  70                  75                  80

CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG        288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                 85                  90                  95

AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC        336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC        384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC        432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC        480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC        528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC        576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA        624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG        672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA        720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA        768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT        816
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT        864
Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
        275                 280                 285
```

```
ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC AGA AGG AAG AGT ATC AAA        912
Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys
    290                 295                 300

AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG        960
Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
305                 310                 315                 320

ACT GAG GCC CAC GCT TGAGGTCCCC G                                      986
Thr Glu Ala His Ala
                325
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
  1               5                  10                  15

Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
             20                  25                  30

Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
         35                  40                  45

Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
     50                  55                  60

His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
 65                  70                  75                  80

Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                 85                  90                  95

Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175
```

```
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
        275                 280                 285

Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys
    290                 295                 300

Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
305                 310                 315                 320

Thr Glu Ala His Ala
                325
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2134 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 116..1177

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 203..1177

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GTACTCGCCA CGGCACCCAG GCTGCGCGCA CGCGGTCCCG GTGTGCAGCT GGAGAGCGAG      60

CGGCCACCGG GAGCCCCCGG CACAGCCCGC GCCCGCCCCG CAGGAGCCCG CGAAG ATG      118
                                                              Met
                                                              -29

CCC CGG CGC AGC CTG CAC GCG GCG GCC GTG CTC CTG CTG GTG ATC TTA       166
```

```
Pro Arg Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile Leu
            -25                 -20                 -15

AAG GAA CAG CCT TCC AGC CCG GCC CCA GTG AAC GGT TCC AAG TGG ACT      214
Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp Thr
        -10                 -5                  1

TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG AAG TAC CCG TCG      262
Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro Ser
  5                 10                  15                  20

TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC AGT GAC ATC CTC      310
Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile Leu
                25                  30                  35

CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA GGC TAC AAT CTG      358
Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn Leu
            40                  45                  50

TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC CAT TCA GTG AAG      406
Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val Lys
        55                  60                  65

CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC CAG TCT CGC TAC      454
Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg Tyr
    70                  75                  80

AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG AAT GAC CCG CAC      502
Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro His
 85                  90                  95                  100

GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC GCC GAG CTG CAC      550
Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu His
                105                 110                 115

ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC AGC ACT GCC AGC      598
Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala Ser
            120                 125                 130

AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC ATT GAG ATG GGC      646
Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met Gly
        135                 140                 145

TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC CTT CAA CAT GTA      694
Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His Val
    150                 155                 160

AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC AAC ATT GAA GAG      742
Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu Glu
165                 170                 175                 180

CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC CGG GGG TCC CTG      790
Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser Leu
                185                 190                 195

ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA GTT TTC CGA AAC      838
Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg Asn
            200                 205                 210
```

```
CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG GAG ACA GCC CTG        886
Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala Leu
        215                 220                 225

TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA ATG ATC AAC AAC        934
Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn Asn
    230                 235                 240

TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA TAC ACC TCC TTC        982
Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser Phe
245                 250                 255                 260

TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT CTG GGC ATC ATC       1030
Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile Ile
                265                 270                 275

CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT ATT GTG GTG GTG       1078
Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val Val
            280                 285                 290

GTG TCC ATT TGG CTT TTC AGA AGG AAG AGT ATC AAA AAA GGT GAT AAC       1126
Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp Asn
        295                 300                 305

AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG ACT GAG GCC CAC       1174
Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala His
    310                 315                 320

GCT TGAGGTCCCC GGAGCTCCCG GGCACATCCA GGAAGGACCT TGCTTTGGAC            1227
Ala
325

CCTACACACT TCGGCTCTCT GGACACTTGC GACACCTCAA GGTGTTCTCT GTAGCTCAAT     1287

CTGCAAACAT GCCAGGCCTC AGGGATCCTC TGCTGGGTGC CTCCTTGCCT TGGGACCATG     1347

GCCACCCCAG AGCCATCCGA TCGATGGATG GGATGCACTC TCAGACCAAG CAGCAGGAAT     1407

TCAAAGCTGC TTGCTGTAAC TGTGTGAGAT TGTGAAGTGG TCTGAATTCT GGAATCACAA     1467

ACCAAGCCAT GCTGGTGGGC CATTAATGGT TGGAAAACAC TTTCATCCGG GGCTTTGCCA     1527

GAGCGTGCTT TCAAGTGTCC TGGAAATTCT GCTGCTTCTC CAAGCTTTCA GACAAGAATG     1587

TGCACTCTCT GCTTAGGTTT TGCTTGGGAA ACTCAACTTC TTTCCTCTGG AGACGGGGCA     1647

TCTCCCTCTG ATTTCCTTCT GCTATGACAA AACCTTTAAT CTGCACCTTA CAACTCGGGG     1707

ACAAATGGGG ACAGGAAGGA TCAAGTTGTA GAGAGAAAAA GAAAACAAGA GATATACATT     1767

GTGATATATT AGGGACACTT TCACAGTCCT GTCCTCTGGA TCACAGACAC TGCACAGACC     1827

TTAGGGAATG GCAGGTTCAA GTTCCACTTC TTGGTGGGGA TGAGAAGGGA GAGAGAGCTA     1887

GAGGGACAAA GAGAATGAGA AGACATGGAT GATCTGGGAG AGTCTCACTT TGGAATCAGA     1947

ATTGGAATCA CATTCTGTTT ATCAAGCCAT AATGTAAGGA CAGAATAATA CAATATTAAG     2007
```

TCCAAATCCA ACCTCCTGTC AGTGGAGCAG TTATGTTTTA TACTCTACAG ATTTTACAAA 2067

TAATGAGGCT GTTCCTTGAA AATGTGTTGT TGCTGTGTCC TGGAGGAGAC ATGAGTTCCG 2127

AGATGAC 2134

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Pro Arg Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile
-29             -25                 -20                 -15

Leu Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp
           -10                 -5                  1

Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro
      5                  10                  15

Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile
 20                  25                  30                  35

Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn
                40                  45                  50

Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val
            55                  60                  65

Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg
         70                  75                  80

Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro
     85                  90                  95

His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu
100                 105                 110                 115

His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala
                120                 125                 130

Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met
            135                 140                 145

Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His
        150                 155                 160

Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu
    165                 170                 175
```

```
Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser
180                 185                 190                 195

Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg
                200                 205                 210

Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala
            215                 220                 225

Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn
        230                 235                 240

Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser
    245                 250                 255

Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile
260                 265                 270                 275

Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val
                280                 285                 290

Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp
            295                 300                 305

Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala
        310                 315                 320

His Ala
    325
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 624 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CCAATCTGCC TTTGAATCTG GAGGAAATAG GCAGAAACAA AATGACTGTA GAACTTATTC      60

TCTGTAGGCC AAATTTCATT TCAGCCACTT CTGCAGGATC CCTACTGCCA ACCTGGAATG     120

GAGACTTTTA TCTACTTCTC TCTCTCTGAA GATGTCAAAT CGTGGTTTAG ATCAAATATA     180

TTTCAAGCTA TAAAAGCAGG AGGTTATCTG TGCAGGGGGC TGGCATCATG TATTTAGGGG     240

CAAGTAATAA TGGAATGCTA CTAAGATACT CCATATTCTT CCCCGAATCA CACAGACAGT     300

TTCTGACAGG CGCAACTCCT CCATTTTCCT CCCGCAGGTG AGAACCCTGT GGAGATGAGT     360

CAGTGCCATG ACTGAGAAGG AACCGACCCC TAGTTGAGAG CACCTTGCAG TTCCCCGAGA     420

ACTTTCTGAT TCACAGTCTC ATTTTGACAG CATGAAATGT CCTCTTGAAG CATAGCTTTT     480
```

TAAATATCTT TTTCCTTCTA CTCCTCCCTC TGACTCTAAG AATTCTCTCT TCTGGAATCG 540

CTTGAACCCA GGAGGCGGAG GTTGCAGTAA GCCAAGGTCA TGCCACTGCA CTCTAGCCTG 600

GGTGACAGAG CGAGACTCCA TCTC 624

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGA AGG AAG AGT 12
Arg Arg Lys Ser

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Arg Lys Ser
1

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGTCGACG 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATTCGTCGA CTCA 14

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 813 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..813

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG      48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
  1               5                  10                  15

AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC      96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
             20                  25                  30

AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA     144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
         35                  40                  45

GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC     192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
     50                  55                  60
```

```
CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC        240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
 65                  70                  75                  80

CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG        288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                 85                  90                  95

AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC        336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC        384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC        432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC        480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC        528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC        576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA        624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG        672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA        720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA        768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG            813
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu
            260                 265                 270
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 271 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
 1               5                  10                  15

Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
            20                  25                  30

Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
        35                  40                  45

Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
    50                  55                  60

His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
65                  70                  75                  80

Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                85                  90                  95

Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu
            260                 265                 270
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..822

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG          48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
 1               5                  10                  15

AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC          96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
            20                  25                  30

AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA         144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
        35                  40                  45

GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC         192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
    50                  55                  60

CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC         240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
65                  70                  75                  80

CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG         288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                85                  90                  95

AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC         336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC         384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC         432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC         480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160
```

```
CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC      528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC      576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA      624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG      672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA      720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA      768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT      816
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

CTG GGC                                                             822
Leu Gly
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 274 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
  1               5                  10                  15

Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
            20                  25                  30

Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
        35                  40                  45

Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
    50                  55                  60

His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
65                  70                  75                  80
```

```
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                 85                  90                  95

Asn Asp Pro His Gly Ser Glu His Thr Val S r Gly Gln His Phe Ala
            100                 105                 110

Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

Leu Gly
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
CTTTTTTGAT ACCCTTCCTT CTGAA                                    25
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 986 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..975

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG      48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
 1               5                  10                  15

AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC      96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
            20                  25                  30

AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA     144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
        35                  40                  45

GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC     192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
    50                  55                  60

CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC     240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
65                  70                  75                  80

CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG     288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                85                  90                  95

AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC     336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC     384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC     432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC     480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160
```

```
CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC      528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC      576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA      624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG      672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA      720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA      768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT      816
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT      864
Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
        275                 280                 280

ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC AGA AGG AAG GGT ATC AAA      912
Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Gly Ile Lys
    290                 295                 300

AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG      960
Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
305                 310                 315                 320

ACT GAG GCC CAC GCT TGAGGTCCCC G                                    986
Thr Glu Ala His Ala
                325
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
  1               5                  10                  15
```

```
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
                20                  25                  30

Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
            35                  40                  45

Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
        50                  55                  60

His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
65                  70                  75                  80

Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
                85                  90                  95

Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
            100                 105                 110

Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
        115                 120                 125

Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
    130                 135                 140

Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
145                 150                 155                 160

Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
                165                 170                 175

Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
            180                 185                 190

Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
        195                 200                 205

Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
    210                 215                 220

Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
225                 230                 235                 240

Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
                245                 250                 255

Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
            260                 265                 270

Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
        275                 280                 285

Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Gly Ile Lys
    290                 295                 300

Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
305                 310                 315                 320
```

Thr Glu Ala His Ala
325

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACATTGAAGA GCTGCTTCCG G 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AATTTGCACG GGGTTTCGG 19

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTGACACCAC TCAGACCGTG TGTGATCTGG CTCAACCAGT TCTGCGATCC CACCCAGGAA 60

CAGAAGACTG CAAGAAAACG TTACTTCAAC CCCCCTGTGA TCCCATCTGC AACCTGACCA 120

ATCAGCACTC CCCAAGTCCC AAGCCCCTAT CTGCCAAATT ATCTTTAAAA ACTCCCCAGA 180

GGCAGGGTGC AGTGGTTCAA CGCCTGTAAT CCCAGCACTT TAGGTGGATC ACGAGATCAA 240

GAGATCAAGA CCAGCCTGGC CAACATGGTG AAACCCCGTC TTCTTACTAA AAATACAAAA 300

ATTAGCTGGG TGTGGCGGCG CGTGCCTGTA ATCCCAGCTA CCCAGGAGGC TGAGGCAGGA 360

GAATCGCTTG AACCCGTGAG GCAGAGGTTG CAGTGAGCCA AGACCATGCC ACTGCATTTC 420

AGCCTGGGCG ACAGAGGGGA ACTCCGTCTG AACAAACAAA CAAACAAACA ACTCCCGGAA 480

TGCTTGGGGA GACTGATTTG AGTACTGGAA TCCCAGTACT TTAGGAGGCC AAGGTAGGTG 540

GATCATTTGA GGTCAGGAGT TCCAGACCAG CCTGGCCAAC ATGGTGAAAC CCCGTCTCTA 600

CTAAAATTAG AAAAATTAGC CGGGTGTGGT GGTGGGCGCC TGTAATCCCA GCACTTTGGG 660

AAGCCAAGGC AGGTGAATTA TCTGAGGTCG GGAGTTTAAG GCCAGCCTTA AACTGGCGAA 720

ACCCCGCCTC TACTAAAAAT ACAAAAATTA TCTGGGCATG GTGGCATGTG CCTGTAATCC 780

CAGCTACTCG GGAGGCTGAG GCAGGAGAAT CGCTTGAACC CGGGAGGCGG AGGTTGCAGT 840

GAGCCGAGAT CACGCTATTG CACTCCGGCC TGGGCAACAG AGCGAGACTC CGTCTCAAAC 900

AAACAAACAA AGGAACGAAA ACTCCGGTCT CCGGCACGGC AAGCTCTGCG TGAATTACTT 960

TCTCCATTGC AACTCCCCTG TCTTGATAAA TGGGCTCTGT CTAAGCAGCG GGCAAGGTGA 1020

ACTCGTTGGG CTGTTACAGG ACCAGTGACA GACCAAGGCA TGCCACTGAA GGAATCCCTA 1080

GACGCACCCT TCTGGATGTG AGGCAGGCGG ATCTCACCCC ACGCCTGCCA GCAGCTCCTC 1140

GGAGAACTGT GTTCCTGGGT CAGCCCTGGC CCAGAGGAGC GCCGGGGACC CGCAGAGTGC 1200

TGCTGAAGTC AAGGCTACAA CTCACCTAGG ATCTGGGGCG CCAGCCTCCG GTGGGCAGGG 1260

CGTTCTCCTC CCCCACCCCC TCCCCGCACG ATGACATCAA GTGTTTGGCG TTGAGTTGCT 1320

CCATAAAAGC TGCCCGGGGA AGCCAGGAGA GCGAAGGGCG GAC 1363

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTCCACTTGG ATCCGTTCAC TGG 23

WE CLAIM:

1. A substantially purified nucleic acid encoding the amino acid sequence of HCAVIII depicted in SEQ ID NO:2.

2. The nucleic acid of Claim 1 wherein said nucleic acid is mRNA.

3. A cDNA encoding the amino acid sequence of HCAVIII or a portion thereof.

4. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the coding region of the nucleotide sequence depicted in SEQ ID NO:1.

5. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:2.

6. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the coding region of the nucleotide sequence depicted in SEQ ID NO:3.

7. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:4.

8. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the nucleotide sequence depicted in SEQ ID NO:12.

9. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:13.

10. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the nucleotide sequence depicted in SEQ ID NO:14.

11. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:15.

12. The cDNA of Claim 3 comprising the nucleotide sequence depicted in SEQ ID NO:5.

13. The cDNA of Claim 3 comprising the nucleotide sequences depicted in SEQ ID NO:5 and SEQ ID NO:7.

14. A cDNA encoding the amino acid sequence of HCAVIII wherein the phosphorylation region has been mutated.

15. The cDNA of Claim 14 wherein the amino acid sequence is encoded by the nucleic acid sequence depicted in SEQ ID NO:17.

16. The cDNA of Claim 14 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:18.

17. A protein comprising the amino acid sequence of HCAVIII or a portion thereof.

18. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:1.

19. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:2.

20. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:3.

21. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:4.

22. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:12.

23. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:13.

24. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:14.

25. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:15.

26. A protein comprising the amino acid sequence of HCAVIII wherein the phosphorylation region has been mutated.

27. The protein of claim 26 wherein the amino acid sequence is encoded by the nucleic acid sequence depicted in SEQ ID NO:17.

28. The protein of Claim 26 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:18.

29. A recombinant DNA clone comprising a cDNA of a HCAVIII transcript isolatable from human A549 cells of about 1.1 kilobases.

30. An expression vector comprising the nucleic sequence for HCAVIII or a portion thereof.

31. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:1.

32. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:3

33. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:12.

34. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:14.

35. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the nucleotide sequence depicted in SEQ ID NO:17.

36. A method of detecting cancerous and precancerous lung tissue comprising:

(a) preparing a section of biopsy tissue;

(b) probing said tissue with a labeled probe complementary to the cDNA of SEQ ID NO:1;

(c) removing said probe which has not hybridized to the tissue; and

(d) detecting the presence of the hybridized probe.

37. A method for detecting lung cancer antigen specific for non-small cell carcinoma in a human cell specimen comprising:

a) labeling a DNA probe comprising the genomic clone of HCAVIII;

b) reacting the labeled DNA probe with a human test cell specimen and a normal human cell specimen under conditions suitable for hybridization of the labeled probe to any HCAVIII mRNA which may be present in the test and normal cell specimen;

c) removing unreacted components from the test and said normal cell specimens;

d) detecting the hybridized probe bound to the test and normal cell specimens;

e) quantifying and comparing the amount of hybridized probe bound to the test and normal cell specimens.

38. The method of claim 37 further comprising:

a) labeling a DNA probe comprising the genomic clone of HCAVIII with a substrate which can bind to a detecting substance to form a labeled DNA probe;

b) reacting the labeled DNA probe with a human test cell specimen and a normal human cell specimen under conditions suitable for hybridization of the labeled probe to any HCAVIII mRNA which may be present in the test and normal cell specimens;

c) removing unreacted components from the test and normal cell specimens;

d) reacting the test and normal cell specimens with a detecting substance which is capable of fluorescing;

e) comparing the fluorescence of the test and normal cell specimens.

39. A method for screening human specimens for HCAVIII protein, comprising:

a) mixing a human test specimen with a first amount of an antibody specific for the HCAVIII protein in a first reaction well;

b) mixing a control lung cancer antigen comprising at least a portion of the HCAVIII protein with a second amount of said antibody specific for the HCAVIII protein in a second reaction well; and

c) detecting whether said test specimen binds to said antibody as compared to said control lung cancer antigen.

40. A method for testing a human cell sample for lung cancer comprising assaying a cell homogenate for carbonic anhydrase activity.

41. An antibody made by immunizing animals with a lung cancer antigen associated with non-small cell lung cancer cells.

42. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:2.

43. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:4.

44. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:13.

45. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:15.

46. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:18.

47. A therapeutic composition for the treatment of non-small cell lung cancer comprising an antibody to HCAVIII protein bound to a substance which affects the ability of said cancer to replicate.

48. The method of claim 47 wherein said substance is a cancer drug.

49. The method of claim 48 wherein said substance is a radioisotope.

50. The method of claim 49 wherein said substance affects gene expression of a gene encoding HCAVIII.

51. A substantially purified nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO:7.

52. A cDNA comprising the nucleotide sequence depicted in SEQ ID NO:7.

53. A substantially purified nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO:21.

AMENDED CLAIMS

[received by the International Bureau on 20 November 1995 (20.11.95); original claim 41 amended; remaining claims unchanged (1 page)]

41. An antibody made by immunizing animals with HCAVIII, a lung cancer antigen associated with non-small cell lung cancer cells.

42. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:2.

43. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:4.

44. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:13.

45. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:15.

46. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:18.

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HCAI      ASPDWGYDDKNGPE-QWSKLYPIA-NGN----NQSPVDIKTSETKHDTSLKPISVS-YNPATAKE--IINVGHSFHVNFEDNDN
HCAII     -SHHWGYGKHNGPE-HWHKDFPIA-KGE----RQSPVDIDTHTAKYDPSLKPLSVS-YDQATSLR--ILNNGHAFNVEFDDSQD
HCAIII    -AKEWGYASHNGPD-HWHELFPNA-KGE----NQSPVELHTKDIRHDPSLQPWSVS-YDGGSAKT--ILNNGKTCRVVFDDTYD
HCAIV     AESHWCYEVQAESS-NYPCLVPVKWGGNCQKDRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWT--VQNNGHSVMMLLEN--K
HCAVI    QHVSDWTYSEGALDEAHWPQHYPAC-GGQ----RQSPINLQRTKVRYNPSLKGLNMTGYETQAGEFP-MVNNGHTVQIGLPSTMR
HCAVII    GHHGWGYGQ-DDGPSHWHKLYPIA-QG----DRQSPINIISSQAVYSPSLQPLELS-YEACMSLS--ITNNGHSVQVDFNDSDD
HCAV      --CAWQTSNNTLHP-LWTVPVSVP-GGT----RQSPINIQWRDSVYDPQLKPLRVS-YEAASCLY--IWNTGYLFQVEFDDATE
HCAVIII   --SKWTYFGPDGEN-SWSKKYPSC-GGL----LQSPIDLHSDILQYDASLTPLEFQGYNLSANKQFLLTNNGHSVKLNLP-S-D

HCAI      RSVLKGGPFSDSYRLFQFHFHWG--STNEHGSEHTVDGVKYSAELHVAH-WNSAKYSSLAEAASKADGLAVIGVLM--KVG-EA
HCAII     KAVLKGGPLDGTYRLIQFHFHWG--SLDGQGSEHTVDKKKYAAELHLVH-WNT-KYGDFGKAVQQPDGLAVLGIFL--KVG-SA
HCAIII    RSMLRGGPLPGPYRLRQFHLHWG--SSDDHGSEHTVDGVKYAAELHLVH-WNP-KYNTFKEALKQRDGIAVIGIFL--KIG-HE
HCAIV     ASISGGG-LPAPYQAKQLHLHWS--DLPYKGSEHSLDGEHFAMEMHIVHEKEKGTSRNVKEAQDPEDEIAVLAFLV--EAGTQV
HCAVI     MTVA-DG---IVYIAQQMHFHWGGASSEISGSEHTVDGIRHVIEIHIVH-YNS-KYKTYDIAQDAPDGLAVLAAFVEVKNY-PE
HCAVII    RTVVTGGPLEGPYRLKQFHFHWG--KKHDVGSEHTVDGKSFPSELHLVH-WNAKKYSTFGEAASAPDGLAVVGVFL--ETG-DE
HCAV      ASGISGGPLENHYRLKQFHFHWG--AVNEGGSEHTVDGHAYPAELHLVH-WNSVKYQNYKEAVVGENGLAVIGVFL--KLG-AH
HCAVIII   --MHIQG-LQSRYSATQLHLHWG-NPNDPHGSEHTVSGQHFAAELHIVH-YNSDLYPDASTASNKSEGLAVLAVLI--EMG-SF

HCAI      NPKLQKVLDALQAIKTKGKRAPFTNFDPSTLLPSSL---DFWTYPGSLTHPPLYESVTWIICKESISVSSEQLAQF-RSLLSNV
HCAII     KPGLQKVVDVLDSIKTKGKSADFTNFDPRGLLPESL---DYWTYPGSLTTPPLLECVTWIVLKEPISVSSEQVLKF-RKLNFNG
HCAIII    NGEFQIFLDALDKIKTKGKEAPFTKFDPSCLFPACR---DYWTYQGSFTTPPCEECIVWLLLKEPMTVSSDQMAKL-RSLLSSA
HCAIV     NEGFQPLVEALSNIPKPEMSTTMAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVFREPIQLHREQILAFSQKL--YY
HCAVI     NTYYSNFISHLANIKYPGQRTTLTGLDVQDMLPRNLQ--HYYTYHGSLTTPPCTENVHWFVLADFVKLSRTQVWKLENSLLDHR
HCAVII    HPSMNRLTDALYMVRFKGTKAQFSCFNPKCLLPAS---RHYWTYPGSLTTPPLSESVTWIVLREPICISERQMGKF-RSLLFTS
HCAV      HQTLQRLVDILPEIKHKDARAAMRPFDPSTLLPTCW---DYWTYAGSLTTPPLTESVTWIIQKEPVEVAPSQLSAF-RTLLFSA
HCAVIII   NPSYDKIFSHLQHVKYKGQEAFVPGFNIEELLPERT--AEYYRYRGSLTTPPCNPTVLWTVFRNPVQISQEQLLALETALYCTH

HCAI      EGDNAVPMQHNN-RPTQPLKGRTVRASF
HCAII     EGEPEELMVDNW-RPAQPLKNRQIKASFK
HCAIII    ENEPPVPLVSNW-RPPQPINNRVVRASFK
HCAIV     DKEQTVSMKDNV-RPLQQLGQRTVIKSGAPGRPLPWALPALLGPMLACLLAGFLR
HCAVI     NKTIH------NDYRRTQPLNHRVVE-SNFPNQEYTLGSEFQFYLHKIEEILDYLRRALN
HCAVII    EDDERI-HMVNNFRPPQPLKGRVVKASFRA
HCAV      LGEEEK-MMVNNYRPLQPLMNRKVWASFQATNEGTRS
HCAVIII   MDDPSPREMINNFRQVQKFDERLVYTSFSQVQVCTAAGLSLGIILSLALAGILGICIVVVVSIWLFRRKSIKKGDNKGVIYKPA

HCAVIII   TKMETEAHA*
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INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US95/09145

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/22.1, 23.1; 530/350, 387.1; 435/4, 6, 7.1, 7.2; 514/44, 424/85.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	American Journal of Pathology, Volume 142, Number 1, issued January 1993, Kim et al, "Interphase cytogenetics in paraffin sections of lung tumors by non-isotopic in situ hybridization", pages 307-317.	36-38
A	Cancer (supplement), Volume 69, Number 6, issued 15 March 1992, Gray et al, "Molecular Cytogenetics in Human Cancer Diagnosis", pages 1536-1542.	36-38
A	Cancer Research (Supplement), Volume 52, issue 01 May 1992, Birrer et al, "Application of molecular genetics to the early diagnosis and screening of lung cancer", pages 2658s-2664s.	36-40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
12 OCTOBER 1995

Date of mailing of the international search report
25OCT1995

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INTERNATIONAL SEARCH REPORT

Int...national application No.
PCT/US95/09145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 5,134,075 (HELLSTROM ET AL) 28 July 1992, especially column 4 lines 34-64.	39, 41, 47-50
Y	US,A, 4,816,402 (ROSEN ET AL) 28 March 1989, see entire document.	39, 41
A	Gastroenterology, Volume 105, Number 3, issued 1993, Mori et al, "The significance of carbonic anhydrase expression in human colorectal cancer", pages 820-826, see abstract.	40
A	DNA and Cell Biology, Volume 11, Number 7, issued September 1992, Skonier et al, "cDNA cloning and sequence analysis of Big-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta", pages 511-522, see entire document.	1-35, 42-46, 51-53

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US95/09145

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US95/09145

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 19/00, 21/00, 21/02, 21/04; C07K 1/00, 14/00, 17/00, 16/00; C12Q 1/00, 1/68; G01N 33/53, 33/567; A01N 43/04; A61K 31/70

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/22.1, 23.1; 530/350, 387.1; 435/4, 6, 7.1, 7.2; 514/44, 424/85.8

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CAPLUS, CANCERLIT
search terms: A549, HCAVIII, Human Cancer Antigen VIII, Cell surface antigen, Cell surface marker, Non-small cell lung cancer, Carbonic Anhydrase

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-39 and 41-53, drawn to nucleic acids encoding the amino acid sequence of HCVIII, the protein expressed thereof, antibodies to the proteins and methods using one of the above.

Group II, claim(s) 40, drawn to a method for testing a human cell sample for lung cancer by assaying a cell homogenate for carbonic anhydrase activity.

Pursuant to 37 CFR § 1.475(d) the additional method(s) beyond the one first method of use are considered to lack unity and are properly separated.